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(54) Title: TARCH CAPSULES CONTAINING MICROORGANISMS AND/OR POLYPEPTIDES OR PROTEINS AND A PROCESS FOR PRODUCING THEM

(57) Abstract

The invention relates to starch capsules which protect various substances, such as living microbes or enzymes, against the effect of the environment or the intestines, and to a method for manufacturing such capsules. A fraction of a suitable size category is chosen from the starch granules, the porosity of the granules is improved by hydrolyzing, and the granules are filled with desired substances, such as living microbes and/or enzymes. When desired, the starch granules can be coated with a suitable biopolymer, such as starch or amylose.

Starch capsules containing micro-organisms and/or polypeptides or proteins and a process for producing them.

The present invention relates to starch capsules. The invention specifically relates to starch capsules containing micro-organisms and/or polypeptides or proteins and a method for manufacturing such starch capsules.

Microbes that are added to foodstuffs and contribute to human health by improving the balance of microbes in the intestines are called probiotes. The probiotic effect of lactic acid bacteria, such as *Lactobacillus, Streptococcus, Pediococcus, Lactococcus, Leuconostoc, Corynebacterium, Enterococcus* and *Bifidobacterium* on human nutrition is well known. The task of the microbes inherently in the human intestine is to prevent the growth of detrimental bacteria and the generation of various inflammations. It has been observed that probiotes also prevent the generation of intestinal cancers. It has been discovered that foodstuffs that contain probiotes increase the tolerance to lactose of people who cannot consume milk products. In connection with various diseases or, for example, treatment with antibiotics, the useful microbe population of the intestines can be destroyed. Quick restoration can be accomplished by consuming products containing lactic acid bacteria. Such products include various sour milk products or vegetable or corn products leavened with lactic acid bacteria. Orally-taken lactic acid bacteria preparations are commercially available in the form of capsules, tablets or powders. However, probiotes are easily destroyed in acidic conditions. The majority of the probiotes consumed are destroyed already in the upper part of the alimentary tract under the effect of low pH (pH 2) or bile acids. Even if the lactic acid bacteria preparations as such contained proper lactic acid bacteria populations which could effectively restore the microbial balance in the intestines, the majority of the lactic acid bacteria dies before [illegible] intestines all the way to the large intestine. Therefore, one should consume foodstuffs containing lactic acid bacteria or lactic acid bacteria preparations for long periods of time [illegible] large quantities [illegible] they [illegible] the desired effect.

The stability of lactic acid bacteria on the pharmacy or shop shelf also constitutes a problem. The microbes should be protected against the effect of oxygen and the fluctuations in humidity and temperature. Otherwise, the lactic acid bacteria content of the lactic acid bacteria preparations is so low already when they are bought that the product will not have the desired effect when consumed.

Efforts have been made to protect living microbes with various packaging methods by packing the microbes in capsules or tablets so that the surface of the capsule or the tablet melts under the effect of humidity or at a certain pH value. Other methods include, for example, freeze-drying. However, suitable conditions must be sought for each group of microbes before freeze-drying, such as a culture medium, the concentration of cells, protective material, pH, humidity, speed of cooling, primary and secondary drying time, the method of closing the containers, etc. Conservation in a vacuum or a gaseous atmosphere improves the stability of cells protected by freeze-drying. The optimal conditions for storage life mean protection against light and fluctuations in humidity as well as a low storage temperature. An increase in the storage temperature increases the inhibition of the cells. For example, freeze-dried starter cultures should be kept at a temperature of -40 ..-20°C. It has been discovered that the rehydration conditions of freeze-dried cells, such as the temperature and the composition of the solution, has a crucial effect on restoring the functionality of the cultures.

Should the stability of probiotes in products and on the shop shelf not constitute a problem, probiotes could also be added to various - mostly dry - foodstuffs, such as grain products, muesli, and sweets.

It is an aim of the present invention is to provide a certain kind of a protective package

The purpose of the protective package is to protect living microbes so that the microbes

foodstuffs. Another purpose of the protective package is to give protection against the acidity of the stomach and the bile acids and other conditions in the intestines so that the microbes stay alive in the intestines as long as is appropriate from the viewpoint of the microbes' effect.

The present invention is based on the surprising finding that starch granules can be used to protect living microbes.

According to another embodiment of the invention, starch granules can be used to protect various polypeptides and proteins, enzymes in particular, which should keep their activity during storage, conservation, and processing or while in the intestines.

International patent publication WO 89/04842 describes the use of starch granules as a carrier for absorbed functional compositions. The starch granules are treated with alpha-amylase or glucoamylase. The publication suggests that starch granules be used as adjuvants for antiperspirants or as bulking agents for foods and drinks. It suggests that liquid substances be formulated, by using treated starch granules, into a powder, paste or cream formulation which is easier to pack or otherwise more practical. To strengthen the structure of hydrolyzed starch granules, the publication suggests treatment of the starch with cross-linking agents, such as sodium trimetaphosphate. If the substance to be absorbed into the starch granules has a lipid character, the starch matrix can, according to the publication, be treated with substances that render the pore surfaces more lipophilic. Such substances include, for example, synthetic polymers such as methylcellulose. The publication mentions that the substances to be absorbed can be, for example, salad oils, flavours, insect repellents, insecticides, herbicides, perfumes, moisturizers, soaps, waxes, body creams and lotions, vitamins and therapeutic drugs.

[illegible]

[illegible] of mechanical compression decomposition, by disintegration of the binding agents or other substances, or by dissolution or diffusion from the porous surface. The diameter of the [illegible]

suggests the use of aggregates in formulating orally dosed pharmaceutical compounds, among others, so that the formulation protects the active ingredients against the acidic and digesting conditions of the stomach and that the active ingredients would not be released until the small intestine. The aggregates were prepared by suspending the starch granules in a suitable solution containing binding agents and by spray drying the suspension. According to the publication, the aggregates could be coated with a polymer after carrying the functional compounds inside the aggregates. The binding agents could be biodegradable polymers, such as polysaccharides (gums originating from algae or plants, pectins, agar-agar, alginate, gelatine, dextrin, starch derivatives) and cellulose bearing substances, such as carboxy-methyl cellulose, hydroxy-methyl cellulose, hydroxy-propyl cellulose, etc., proteins, particularly those which are not inherently present in starch granules, as well as polyesters. The polymers could also be non-biodegradable, synthetic or semisynthetic, such as polyvinyl alcohol poly-N-vinyl-2-pyrrolidone or polymers or copolymers of acrylic or methacrylic acid or their amide derivatives, such as polyacrylamide. The coating substances could be the same or different polymers than the binding agents. The functional substances that could be absorbed into the aggregates could be the same substances as listed in application WO 89/04842.

U.S. Patent No. 4,551,177 describes a compressible starch that can be used as a binder for tablets. Cold-water-insoluble granular starch was treated with acid, alkali or alpha-amylase, whereby altered, weakened granules were obtained which, when compressed, showed a good binding capacity.

Patent publication EP 0 539 910 A1 describes the treatment of starch granules with an amylase. The intention was to modify the boiling viscosity of the starch granules. The patent publication suggests that the amylase-treated starch granules be used in instant liquid food, for example, or as mixed with non-treated starch granules, whereby blends of starch granules having various viscosity values can be obtained. According to the

granules are able to adsorb oils.

describes the use of amylose films to protect pharmaceutical preparations. According to the publication, insoluble polymers, such as ethyl cellulose, were used to control the swelling and decomposition of the amylose and, thus, the releasing speed of the medicine in conditions that simulated the conditions of the alimentary tract. According to the publication, the release of the medicine from the preparation was due to the decomposition of the amylose component of the compound under the effect of bacterial enzymes and not because of mechanical decomposition of the coating.

Solutions of the prior art technology do not suggest a good method for the protection of micro-organisms or polypeptides or proteins, such as enzymes, against the effects of the environment. Prior art publications, such as the publications WO 89/04842 and U.S. 5,670,490, suggest that various functional substances be protected by using starch granules but they do not suggest that micro-organisms or proteins be protected. In the formation of aggregates described in U.S. Patent No. 5,670,490, various binding agents are used, for example, various polymers which can be detrimental when introduced into the organs or during the manufacturing process.

The purpose of the present invention is to eliminate the disadvantages of the known technology and to provide a whole new method which advantageously employs natural polymers. By using the method, living micro-organisms and/or polypeptides or proteins can be protected against the effects of the environment during storage or in the human or animal intestines.

In the method according to the invention, the starch granules are hydrolyzed so that the surface structure becomes perforated and the inside porous, whereby the internal space of the granule forms a hollow, capsule-like space. The capsule-type space can be filled with micro-organisms and/or polypeptides or proteins and the surface of the granule can be [illegible] starch dissolved in hot water or a component of the starch, an amylose, or by closing the pores on the surface of the starch granule by smaller starch granules of a suitable size.

The present invention is based on the perception that starch is hydrolyzed with amylolytic enzymes, such as alpha-amylases, ß-amylases or glucoamylases. The amorphic components of the granule are hydrolyzed and the crystalline areas remain. These crystalline components are fairly stable also in the human alimentary tract. After hydrolyzation, the starch granule is filled with the desired substances, such as living micro-organisms or polypeptides or proteins, such as enzymes or a mixture thereof. Microbe cultures may inherently contain certain enzymes produced by microbes. Microbes can also produce the hydrolytic enzymes needed in the hydrolysation of a starch granule, when allowed to grow inside the starch granules in suitable conditions.

When so desired, the starch can be coated with natural biopolymers, such as cellulose, pectins, proteins, preferably with starch. The starch, one of its components, a linear amylose in particular, is capable of film forming. The starch can be modified by physical means (for example, by means of temperature) so that it becomes more stable and more resistant against the liquids of the stomach and the small intestine. The various techniques for coating starch granules include spraying with a starch/amylose solution or mixing the granules with a starch/amylose solution and allowing the starch to crystallize onto the surface of the granule. The starch/amylose solution can also be precipitated onto the surface of the granules by using ethanol. According to one embodiment of the invention, the pores on the surface of the granule can be closed with suitably small starch granules. Instead of the starch/amylose, the coating of starch granules can be carried out by employing other biopolymers, if their film forming properties and dissolving properties are as good as those of the starch and the amylose.

The starch granules can be filled with living microbes, such as lactic acid bacteria, or with starters used by the food industry or with polypeptides or proteins, such as enzymes. It is preferable to use the method according to the invention, for example, for encapsulating various industrial enzymes (textile and food enzymes and those used by the wood-

fibres.

origins deviate as to their size and composition. These differences can be utilized in different applications.

One object of the invention is starch capsules which comprise starch granules which have a porous structure and are filled with a desired substance, preferably with microbes or microbes and/or polypeptides or proteins, such as enzymes.

Another object of the invention is a method for manufacturing the starch capsules, comprising the following steps:

- selecting starch granules of a suitable size in accordance with the purpose of use,
- improving the porousity of the starch granules by hydrolyzing the starch granules chemically or by using enzymes, and
- filling the granules with micro-organisms or with micro-organisms and polypeptides or proteins or polypeptides or proteins.

When so desired, the granules are coated with a biopolymer, preferably starch.

With the aid of the invention, considerable advantages can be obtained. The starch granules according to the invention keep well at room temperature for several months.

Lactic acid bacteria stored in the starch capsules according to the invention or starters used by the food industry, foodstuffs containing the probiotcs, or enzymes can be kept at room temperature for long periods of time. The quality of preparations containing living microbes, such as lactic acid bacteria preparations, or foodstuffs containing probiotcs is improved when the microbe content of the product is already high from the beginning. The activity and the efficacy of the enzymes improve when the enzyme is not exposed to the effect of fluctuations in humidity, temperature, oxygen or acidity in its environment.

[illegible] not released from the starch capsules too early in the intestines. Similarly, the effect of the enzymes is improved and the duration of action is increased when the activity of the [illegible]

In the following, the present invention is studied more closely with the aid of a detailed description and exemplary embodiments.

Fig. 1. The size distribution of separated starch granules on Coulter.

Fig. 2. A starch granule hydrolyzed with an alpha-amylase.

Fig. 3. A starch granule filled with amylose.

Fig. 4. A starch granule filled with lactic acid bacteria (cut thickness 4µm).

Fig. 5. The growth curve of lactic acid bacteria.

Starch is the reserve polysaccharide of plants. It consists of two polymers of glucose, the linear amylose and the amylopectin that is very branched. The starch granules can be hydrolyzed with amylolytic enzymes, such as alpha-amylases. In that case, the amorphic components of the starch granules are hydrolyzed and the crystalline areas remain. Starches of various origins deviate as to their size and composition.

The starch used in manufacturing the starch capsules is preferably natural starch. It can originate from barley, potato, wheat, oats, pea, corn, tapioca, sago, rice or similar tuber vegetable or corn crop, it preferably originates from potato, barley, wheat or corn, most preferably from potato.

Starch granules of a suitable size can be separated from the starch by suspending the starch in water, by mixing and allowing the starch granules to sediment. The solution and small granules are poured out of the top of the sediment. The sedimentation can be repeated several times (2 to 10 times) and the granules of a desired size thus obtained can be freeze-dried.

The present invention employs starch granules having a size of 10 to 100 µm, preferably 30 to 100 µm, most preferably 50 to 100 µm. It is advantageous to use larger starch granules because, when hydrolyzing, larger cavities are formed in larger granules, whereby

[illegible]

fractionated. Otherwise, the starch granules are fractionated into fractions of various size categories and a suitable starch granule fraction is chosen from the viewpoint of the

[illegible]

microbes, it is preferable to choose large starch granules. Potato starch provides starch granules of a particularly suitable size.

The granules are hydrolyzed either chemically or by using enzymes. The enzymes are preferably alpha-amylases, ß-amylases and/or glucoamylases which typically originate from the *Rhizopus, Aspergillus or Bacillus* genera. Examples of suitable alpha-amylases and ß-amylases include MEGAZYME® (Australia). The pores or holes inherently in starch granules are less than 10nm. When starch granules are hydrolyzed, the size of the holes becomes 1 to 10μm. Holes made by alpha-amylase, for example, are about 1μm.

For hydrolysis, the starch granules are suspended in water to form about a 5-15% solution. The amount of the amylase solution added is 1000 - 10 000 U/g of granules depending on the enzyme product. The hydrolysing is carried out at a temperature that is suitable for the activity of the enzyme but does not alter the structure of the starch, for example, at a temperature of 30-40°C or, alternatively, under high pressure so that the temperature need not be so high. The objective of the hydrolysis is to hydrolyze 3-60%, preferably 30-50%, and most preferably 40% of the dry content of the chosen starch granules.

A suitable amount of starch granules, for example, 1 weight fraction is mixed with 10 to 100 weight fractions of a solution of living bacteria (PFU 10^8 - 10^9) or 1 weight fraction with 10 to 100 weight fractions of an enzyme solution of a suitable concentration or another substance which is to be contained by the starch granules.

According to one preferred embodiment of the invention, hydrolyzed starch granules are filled with a desired substance and freeze-dried.

According to another preferred embodiment of the invention, the hydrolyzed starch granules are freeze-dried, filled with a desired substance and, possibly, freeze-dried again.

with a microbe solution containing amylolytic enzymes, preferably with a growth medium of lactic acid bacteria, in suitable conditions so that the microbes are reproduced

the hydrolysis products released from the starch granules for their nutrition and, at the same time, producing their own metabolic products, such as lactic acid and acetic acid which reduce the pH to a value advantageous to the growth of microbes, and polysaccharides which further stabilize the structure of the starch granules, and enzymes which hydrolyze the starch granules. It is also preferable to add a lactic acid/acetic acid solution to the solution and to regulate the pH of the solution so that it is advantageous to the growth of microbes.

An application could also be considered in which no hydrolyzing enzymes or chemicals are added to the mixture of starch granules and microbes, but the microbes are allowed to produce the enzymes needed in the hydrolysis.

When mixing the starch granules with the microbe solution, a temperature advantageous for the growth of microbes can be chosen, preferably less than 40°C, most preferably 30-37°C, and the mixing time can be long enough for the microbes to reproduce themselves and grow into the porous and hollow inner space of the starch granules.

After hydrolyzing and filling, the starch granules can be separated from the treating solution for various applications and freeze-dried, and cooled down in a deep-freezer or in liquid nitrogen. As a result, a powder is obtained which is easy to process and in which the capsules formed by the starch granules are essentially separate, not forming aggregates. The hydrolyzed starch capsules give protection to living microbes and/or polypeptides or proteins during shelf storage or, for example, in foodstuffs.

When so desired, the filled starch granules can be coated so that the substances enclosed in the starch granules cannot be released prematurely or that the environment has no adverse effect on them. This is advantageous, particularly when the starch capsules are to be used to transport microbes and/or polypeptides or proteins to the intestines. Coating i[illegible]

The coating can be carried out by using a biopolymer which is capable of film forming, preferably a starch and most preferably an amylose. An 0.1 - 70% or 0.1-6% solution in

proportion to the starch can be prepared from the starch or the amylose. The starch or amylose solution can be sprayed onto the surface of the granules so that the starch or amylose concentration is 1-6% of the weight of the granules, and allowed to cool so that the starch/amylose forms a gel on the surface of the granules. In this case, it is preferable to use an 0.1-6%, more preferable an 0.1-2% starch solution. Alternatively, the granules can be mixed with the starch or amylose solution and allowed to crystallize at a low temperature (4-10°C). In this case, it is preferable to use an 0.1-70% starch solution. The starch or amylose solution can also be precipitated on the granule surfaces by using ethanol. According to one alternative, the starch granules can be coated with starch particles of a smaller size than themselves. When hydrolyzing, the smaller starch granules fit into the holes formed on the surface of the starch granules. The size of the starch granules used for the coating is preferably within 1-10 μm.

The starch film coating can be implemented as a water-based coating which is a clear advantage compared with film coating using organic solutions (industrial safety, dissolvent residue, environmental aspects).

We could also consider combining the biopolymer, preferably starch or amylose used for coating the capsules, with various film coating materials used in the pharmacy and accepted pharmaceutically. One protecting film material used in the pharmacy is, for example, hydroxy-propyl methyl cellulose (HPMC), regarding regulating film materials, we could mention ethyl cellulose which could be used to regulate the decomposition speed of the starch films in the alimentary tract. The coating material preferably consists of 50-100%, preferably 90-100% biopolymers, such as starch or amylose, the rest is 0-50 %, preferably 0-10% pharmaceutically accepted film coating materials.

According to the invention, the granules can be filled with micro-organisms, such as various bacteria, yeasts or molds.

Streptococcus, Pediococcus, Lactococcus, Leuconostoc, Corynebacterium, Enterococcus or *Bifidobacterium* or they can be yeasts and belong to the *Saccharomyces* genus.

When preserved in the capsule according to the invention, the liveliness of the microbes is decreased by only 1-10% at 20°C in 2 months, and by only 10-30% in 6 months.

According to the invention, the granules can be filled with various polypeptides or proteins, such as enzymes. The enzymes can be various industrial enzymes, such as those used by the foodstuff, textile, and wood-processing industries, with the purpose of improving the maintenance of the activity of these enzymes during storage and processing. The enzymes are, for example, baking enzymes, the premature action of which is to be prevented. The enzymes can also be enzymes that are used to improve the digestibility and the decomposition of foodstuffs or their components in the intestines, such as the enzymes that decompose lactose.

The following non-limiting examples illustrate the invention.

Example 1

1. Separation of starch granules

Potato starch is suspended in water (a 5% solution). The solution is poured into a glass tube (diameter 4 cm and height 15 cm). The solution is agitated and the granules are allowed to sediment for 8 minutes. Large granules (30-100 μm) sediment on the bottom of the tube. The solution (small granules) is poured from the top of the sediment to another container. The sedimentation is repeated 3 times. The sediment (large granules) is freeze-dried. The sediment is centrifuged and freeze-dried. Fig. 1 shows the size distribution of the separated starch fraction (defined with Coulter).

2. Hydrolysis of the starch with alpha-amylase

was allowed to take place overnight at a temperature of over 30°C in a water bath provided with a magnetic stirrer. The solution was centrifuged and the sediment was freeze-dried.

separated potato starch granules became hydrolyzed. Fig. 2 shows a hydrolyzed starch granule.

3. Filling of hydrolyzed starch granules with lactic acid bacteria

Starch granules (10g) and 100ml of an MRS solution (deMan-Rogose-Sharpe culture medium, Oxoid, Unipath Ltd, Basingstoke, Hampshire, England), in which *Lactobacillus rhamnosus* (ATCC 53109) (10^8 - 10^9 CFU/ml) or *Lactococcus lactis* (VTT E-90414) (10^8 - 10^9 CFU/ml) lactic acid bacteria were cultivated, were combined. The mixtures were kept in a water-bath (30°C) with a magnetic stirrer overnight. The solution was centrifuged out. The sediment was washed with water and the water was centrifuged out. The filled starch granules were freeze-dried. Fig. 4 shows a starch granule filled with lactic acid bacteria.

4. Liveliness of bacteria

The liveliness of the bacteria when fresh at room temperature (20°C) was $3x10^7$ CFU/g and after 2 months storage (in an excicator at 20°C) $3x10^5$ CFU/g. The liveliness of a sample kept in a deep-freezer (-18°C) for 2 months was $2x10^7$ CFU/g.

Example 2

Hydrolyzed starch granules were filled with lactic acid bacteria, as described in sections 1-3 of Example 1. Freeze-dried starch lactic acid bacteria particles were coated with amylose. An 0.1-2% solution was prepared from the amylose by heating it to a temperature of 170°C. The solution was cooled down to 60°C or to 30°C. The particles were sprayed with the solution so that the amylose content was about 1-6% of the particle weight or the particles were mixed with the solution and allowed to crystallize overnight at +4°C. Fig. 3 shows a starch granule coated with amylose.

Starch granules were hydrolyzed and filled simultaneously. Separated large starch granules (10g), lactic acid bacteria in an MRS solution (10[illegible] - 10[illegible] CFU/ml 100 ml), an enzyme [illegible]

of the solution) were mixed. The solution was hydrolyzed overnight at 30°C with a magnetic stirrer. It was discovered that the increase in the lactic acid bacteria content was higher when α-amylase (1= 10 IU/ml, 2=50 IU/ml, 3=100 IU/ml, 4=200 IU/ml, 5=300 IU/ml, PS=control; Fig.5,) was added to the MRS solution. The mixture was centrifuged and the sediment was freeze-dried. The filled particles were coated according to Example 2. The liveliness of the bacteria when fresh was $7x10^9$ CFU/g, $4x10^8$ CFU/g after 1 month storage, and $1x10^9$ CFU/g after 2 months storage, $3.1x10^8$ CFU/g (in an excicator at 20 °C) after 6 months storage. The liveliness of the bacteria stored in a deep-freezer (-18°C) for 6 months was $3.7x10^9$ CFU/g.

Claims

1. A starch capsule, c h a r a c t e r i z e d in that it comprises a starch granule which has a porous structure as a result of hydrolyzing and is filled with micro-organisms or micro-organisms and/or polypeptides or proteins.

2. A starch capsule according to claim 1, c h a r a c t e r i z e d in that the starch granule is hydrolyzed by using enzymes.

3. A starch capsule according to claim 1 or 2, c h a r a c t e r i z e d in that the starch granule is hydrolyzed with α-amylase, ß-amylase and/or glucoamylase.

4. A starch capsule according to any one of claims 1 to 3, c h a r a c t e r i z e d in that the starch granule is coated with a biopolymer.

5. A starch capsule according to any one of claims 1 to 4, c h a r a c t e r i z e d in that the starch granule is coated with cellulose, pectin, protein, starch and/or amylose.

6. A starch capsule according to any one of claims 1 to 5, c h a r a c t e r i z e d in that the starch granule is coated with a mixture of cellulose, pectin, protein, starch and/or amylose and a pharmaceutically acceptable film coating material.

7. A starch capsule according to any one of claims 1 to 6, c h a r a c t e r i z e d in that the starch granule originates from barley, potato, wheat, oats, pea, corn, tapioca, sago, rice or similar tuber vegetable or corn crop, preferably from potato, barley, wheat or corn, most preferably from potato.

8. A starch capsule according to any one of claims 1 to 7, c h a r a c t e r i z e d in that the

9. A starch capsule according to any one of claims 1 to 8, c h a r a c t e r i z e d in that the granule is filled with micro-organisms, such as bacteria, yeasts or molds.

10. A starch capsule according to any one of claims 1 to 9, c h a r a c t e r i z e d in that the starch granule is filled with lactic acid bacteria, such as those belonging to the genera of *Lactobacillus, Streptococcus, Pediococcus, Lactococcus, Leuconostoc, Corynebacterium, Enterococcus,* or those belonging to the *Bifidobacterium* genus.

11. A starch capsule according to any one of claims 1 to 9, c h a r a c t e r i z e d in that the micro-organisms are yeasts and belong to the *Saccharomyces* genus.

12. A starch capsule according to claim 1, c h a r a c t e r i z e d in that the granule is filled with enzymes.

13. A starch capsule according to any one of claims 1 to 12, c h a r a c t e r i z e d in that the starch capsule is coated with a starch solution having a concentration of 0.1-70%, preferably 0.1-6% in proportion to the starch, most preferably 0.1-2% in proportion to the starch.

14. A starch capsule according to any one of claims 1 to 12, c h a r a c t e r i z e d in that the perforated surfaces of the starch granules are filled with starch particles having a size of 1-10 µm.

15. A method for manufacturing filled starch capsules, c h a r a c t e r i z e d in that the method comprises the following steps:

- selecting starch granules of a suitable size in accordance with the purpose of use, and
- hydrolyzing the starch granules so that the structure of the granules becomes porous, and
- filling the starch granules with micro-organisms or micro-organisms and/or polypeptides or proteins.

16. A method according to claim 15, c h a r a c t e r i z e d in that hydrolysis is allowed to

[illegible]

17. A method according to claim 15 or 16, c h a r a c t e r i z e d in that the starch granules

[illegible]

18. A method according to any one of claims 15 to 17, c h a r a c t e r i z e d in that the starch granules are hydrolyzed and filled simultaneously.

19. A method according to any one of claims 15 to 18, c h a r a c t e r i z e d in that living micro-organisms are allowed to grow into the starch granules in the presence of hydrolytic enzymes.

20. A method according to any one of claims 15 to 19, c h a r a c t e r i z e d in that the starch granules are coated by crystallizing or spraying a starch solution onto the surface of the granules.

21. A method according to any one of claims 15 to 20, c h a r a c t e r i z e d in that the starch solution used in crystallization is 0.1-70% in proportion to the starch.

22. A method according to any one of claims 15 to 20, c h a r a c t e r i z e d in that the starch solution used in spraying is 0.1-6%, preferably 0.1-2% in proportion to the starch.

23. A method according to any one of claims 15 to 20, c h a r a c t e r i z e d in that the starch granules are coated by mixing the granules with an 0.1-70% starch solution which, when cooled, forms a gel onto the surface of the granules.

24. A method according to any one of claims 15 to 20, c h a r a c t e r i z e d in that the starch granules are coated by mixing the granules with a starch solution which, when precipitated by using ethanol, forms a gel onto the surface of the granules.

25. A method according to any one of claims 15 to 19, c h a r a c t e r i z e d in that the

26. A method for manufacturing filled starch granules, c h a r a c t e r i z e d in that the method comprises the following steps:

- bringing living micro-organisms which are capable of hydrolyzing the starch in contact with the starch granules, and
- allowing the micro-organisms to produce hydrolytic enzymes and, at the same time, to grow into the starch granules.

27. A method according to claim 26, c h a r a c t e r i z e d in that the starch granules are coated with a biopolymer or a mixture of a biopolymer and pharmaceutically acceptable film coating material.

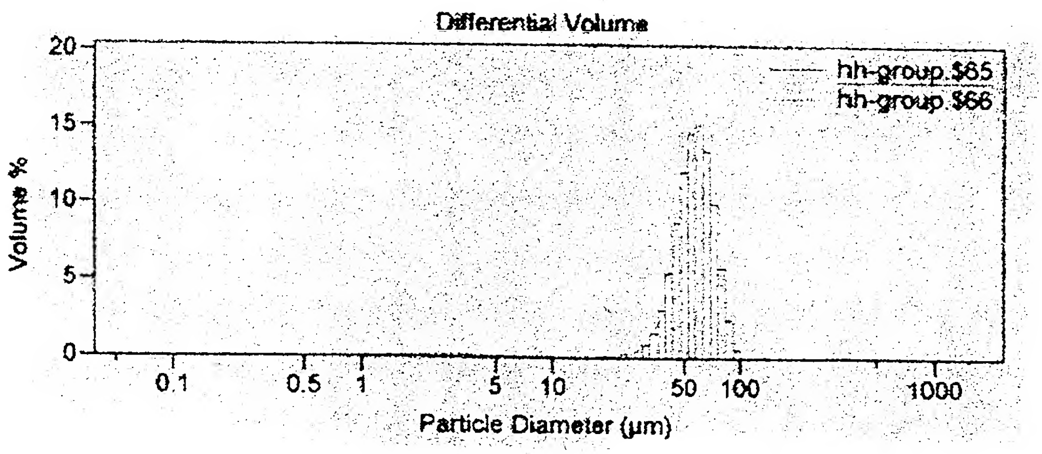
Differential Volume
hh-group.$65
hh-group.$66
Volume %
20
15
10
5
0
0.1
0.5
1
5
10
50
100
1000
Particle Diameter (µm)

Fig. 1

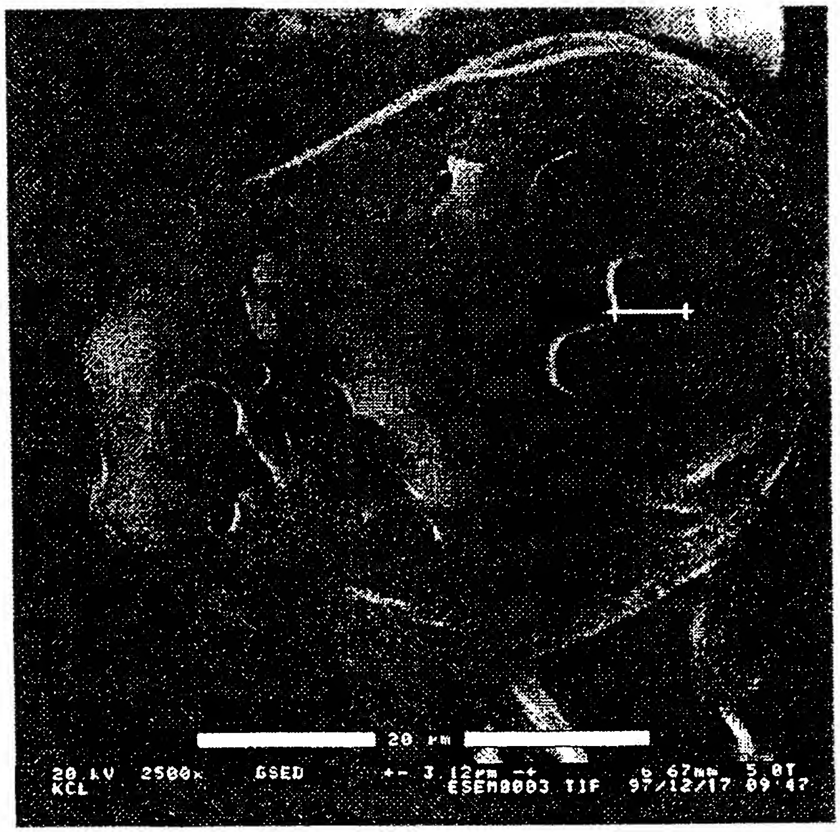
20 µm
20 kV 2500x GSED +- 3.12µm -+ 6.67mm 5.0T
KCL ESEM0003.TIF 97/12/17 09:47

Fig. 2

Fig. 3

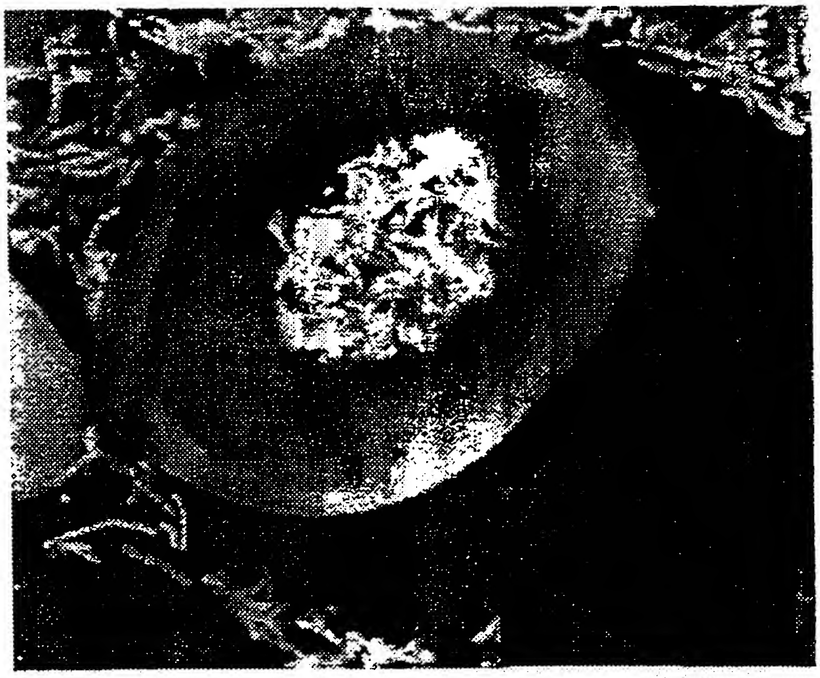

Fig. 4

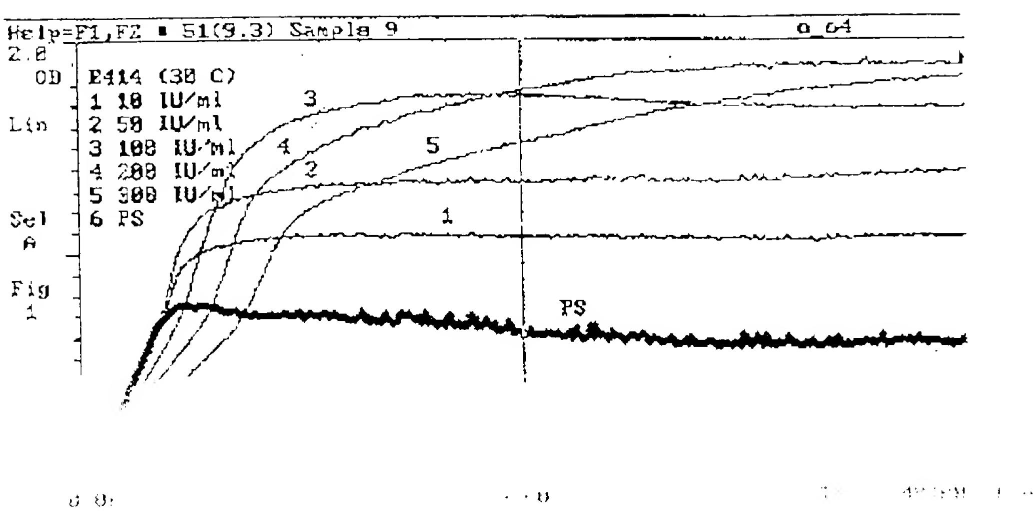
Help=F1,F2 ■ 51(9.3) Sample 9
2.0
OD
Lin
Sel
A
Fig
1
E414 (30 C)
1 10 IU/ml
2 50 IU/ml
3 100 IU/ml
4 200 IU/ml
5 300 IU/ml
6 PS
3
4
5
2
1
PS

Fig. 5

INTERNATIONAL SEARCH REPORT

International application No.
PCT/FI 99/00259

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: A61K 9/52, A61K 9/20, A61K 47/36

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: A61K, C08B

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, EPODOC

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5726161 A (ROY L. WHISTLER), 10 March 1998 (10.03.98), column 3, lines 15-23; column 5, lines 20-34; column 6, lines 8-49; column 7, lines 1-24; claims	1-27
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X	US 4859377 A (BARUCH S. SHASHA ET AL), 22 August 1989 (22.08.89), column 3, line 36 - column 5, line 36; claims	1-14
A		15-27
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[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" erlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance: the claimed invention cannot be [illegible]

[illegible]

26 July 1999	28-07-1999
Name and mailing address of the ISA [illegible]	Authorized officer
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Facsimile No. + 46 8 666 02 86	Telephone No. + 46 8 782 25 [illegible]

INTERNATIONAL SEARCH REPORT

International application No.
PCT/FI 99/00259

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5160745 A (PATRICK P. DELUCA ET AL), 3 November 1992 (03.11.92), column 4, line 52 - column 5, line 10	1-14
A		15-27
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A	WO 9734645 A1 (JOHNSON & JOHNSON MEDICAL, INC.), 25 Sept 1997 (25.09.97), page 3, line 37 - page 4, line 15; claims	1-27
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INTERNATIONAL SEARCH REPORT

Information on patent family members

01/07/99

International application No.
PCT/FI 99/00259

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